Re: More on A.pellucida

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- *From*: "GTO" <gregor_o@xxxxxxxxxxxxxxx>
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Hello, Rene and GR:

I am getting headache from reading this part of the thread. I am convinced that both of you know how to succeed in photomicrography, but this part of the thread entered the state of verbal jousting and looping.

I can only hope that both agree on the following basic summary:

1) Smallest resolvable distance 'd' (diameter of Airy disk) is given by d = 0.61*lambda/NA(obj) assuming we are using a NA(cond) of 1.4 and hence get no limitation due to insufficient illumination.

2) The size of the smallest resolvable distance 'D' in the INTERMEDIATE image (also referred to as real image) is given by D = d * magnification of objective

3) To resolve a feature of the size 'D', one needs a pixel size of D/2 (Nyquist theorem [1]) to avoid undersampling. But remember, this is the ACTIVE pixel size and not the PHYSICAL pixel size in case of a Bayer filter (see point 4). Let's call this the required pixel size 'r'.

4) The required pixel size for a Bayer filter depends now on the wavelength. If blue light is used the active pixel size is 2 times the physical pixel size. For green, it is square−root 2 times the physical pixel size. Let's call a = D/2 to be the active pixel size.

5) To be able to resolve the entire image, the active pixel size must be smaller or equal to the required pixel size: a <= r

Now let's look at an example:

For a Nikon D70 (I don't know the Pentax that GR is using), the active pixel size 'a' is a HUGE 12um when we use primarily blue light (no green). This is much too big to resolve the fine structure with the CCD of a D70 located in the intermediate image plane (direct projection). For an objective with NA 1.40 with lambda = 425nm, d = 0.185 and D = 18.5 um. This gives r = 9.25 um, which is smaller than the 12um provided by the D70 for blue light. When using a 2x projection ocular (or relay lens) the image information is spread out onto a larger area and hence we can multiply 18.5 um by two = 37 um.

Now, r = 18.5um, which is easily captured by the image sensor's pixels with a PHYSICAL size of 6um and using blue light.

Now that would explain one aspect of the fact that GR cannot resolve AP when using direct projection but by using a relay lens. Another important aspect is, of course, the fact that using the appropriate relay lens, one gets a complete correction for aberrations when using older gears.

Cheers,

Gregor

[1] http://en.wikipedia.org/wiki/Nyquist−Shannon_sampling_theorem

"rene" <renevanwezel@xxxxxxxxxxx> wrote in message news:1170089222.158026.197660@xxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
GR, I still find you lacking on all points except for the Baertierchen info.

> You seem confused by the issue of contrast. I will say it one more, final time: contrast increases when the target's image spatial frquencies move to the left on the MTF curve. The only way for this to happen at a fixed pixel or grain size is to decrease the spatial frequency, that is to increase the size of the image.

So what you are suggesting is that if we enlarge the diatom, then we are able to see more. Duh.

René.

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